Effectiveness of methyl bromide as a cargo fumigant for brown treesnakes

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Abstract

The effectiveness of methyl bromide as a fumigant for brown treesnake (*Boiga irregularis*) management was evaluated on Guam. Eighteen snakes in secured cloth bags were randomly positioned in a 47.7-m^3 tarpaulin-covered cargo container for each fumigation treatment. Methyl bromide treatments tested were: $24\,g\,m^{-3}$ and and $12\,g\,m^{-3}$, both for 2-h and 1-h exposures. All treatments, except for $12\,g\,m^{-3}$ for 1 h, resulted in 100% mortality. Two-hour exposures to methyl bromide at 24 and $12\,g\,m^{-3}$ appear to be effective in killing brown treesnakes within reasonable time-frames for shippers under Guam field conditions.
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1. Introduction

The brown treesnake (*Boiga irregularis*) is a significant exotic arboreal nocturnal pest that was probably inadvertently introduced from the Papua New Guinea area to the island of Guam in the late 1940s (Fritts, 1988). This snake was first detected on Guam in the 1950s, became conspicuous in the 1960s and is presently distributed throughout the island with population densities approaching 100 snakes Ha^{-1} in some areas (Rodda and Fritts, 1992a). The brown treesnake is considered to be the primary factor responsible for the extirpation of much of the native vertebrate fauna on Guam (Savidge, 1987; Fritts, 1988; Rodda and Fritts, 1992b). It is mildly venomous and poses a human health risk (Fritts et al., 1990), causes electrical power outages by climbing on wires (Fritts et al., 1987), and preys on domesticated birds (Fritts and McCoid, 1991). Appar-

ently Guam now has the only known reproducing extralimital population of brown treesnakes (Fritts, 1987). Because Guam is a focal point of air and ship cargo traffic in the Pacific, there is the threat that brown treesnakes could be unintentionally introduced to other snake-free islands in the Pacific through cargo shipments. Individual snakes have been discovered in other Pacific regions such as Honolulu, Hawaii; the islands of Wake, Kwajalein, Saipan, Tinian, Rota, and Pohnpei; and Diego Garcia Atoll in the Indian Ocean (Fritts, 1987; McCoid and Stinson, 1991). In May 1993, a brown treesnake was documented on the mainland USA at Ingleside Naval Station near Corpus Christi Bay, Texas, in crated household goods that had arrived from Guam (McCoid et al., 1994).

One method that could be used to reduce the dispersal of snakes is the fumigation of cargo. The present study was designed to test whether the fumigant, methyl bromide, would kill brown tree snakes. Methyl bromide was selected because it is used worldwide as a commodity and quarantine treatment for numerous

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pests. In the United States, the Environmental Protection Agency (EPA) approved labeling directions include low application rates of 4–5 oz 1000 ft^{-3} (4–5 g m^{-3}) for 12- to 18-h exposure times to control rats and mice (Great Lakes Chemical Corporation, 1987). Insect pests have higher application rates (1–5 lb 1000 ft^{-3}, 16–80 g m^{-3}) but exposures as low as 2 h. The US Department of Agriculture Treatment Manual (USDA, 1994) includes many plant, commodity and quarantine treatments for 1.5- to 2-h exposures from 0.5 lb to 3 lb 1000 ft^{-3} (8–48 g m^{-3}). Brock and Howard (1962) reported that methyl bromide killed snakes, but the species were not identified and no data were presented.

2. Materials and methods

Meth-O-Gas$^{®}$ (assay $\geqslant$ 99.8% methyl bromide), manufactured by Great Lakes Chemical Corporation, West Lafayette, Indiana USA, was supplied by No-Ka-Oi Termite and Pest Control Inc., Guam. Fumigation tests were conducted in cargo containers under a tarpaulin using standard US Department of Agriculture (USDA)/Plant Protection and Quarantine (PPQ) treatment procedures as described in the PPQ Treatment Manual, Section 3, Part 1, "Tarpaulin Fumigation—Methyl Bromide," and Part 2, "Aeration Guidelines," (USDA, 1994). In September and October 1991, tests were conducted in the evening between 18:00 and 22:00 h to avoid mid-day temperatures that exceeded 49 °C in the tarpaulin-enclosed containers.

One hundred and eight snakes (mean snout-vent length, SVL, 790 mm, SD = 185 mm) were hand-captured in Guam, weighed, and caged in plastic storage boxes (approx. 5.9 cm long × 3.9 cm wide × 3.5 cm high), 4–5 per box, for 3–28 days before testing. A unique number was assigned to each snake with either an implantable transponder, or permanent ink applied to the head. Snakes were classified as juveniles if the SVL was 900 mm or less, or as adults if the SVL was greater than 900 mm (M. McCoid, Guam Department of Agriculture, Division of Aquatic and Wildlife Resources, personal communication). Snakes were fed once a week; the largest snakes (SVL > 1000 mm) were each fed a laboratory mouse (*Mus musculus*) in individual cages and returned to the group cage after 2–3 days and the smaller fed 2–4 house geckos (*Hemidactylus frenatus*).

Two commercial cargo shipping containers were used under tarpaulins outdoors. The enclosed space under the tarpaulin for each container was 47.7 m^3. Inside dimensions of the cargo containers were 5.9 m long × 2.3 m wide × 2.4 m high. Each container was loaded with non-food cargo, i.e. wood, cardboard boxes and metal drums, to simulate a typical shipping test situation. Six fumigation treatments (T), with methyl bromide (MB) applied m^{-3} enclosed space, were tested: $T1 = 0$ g MB for 2 h (control); $T2 = 24$ g MB for 2 h; $T3 = 24$ g MB for 1 h; $T4 = 12$ g MB for 1 h; $T5 = 24$ g MB for 1 h; and $T6 = 12$ g MB for 2 h.

Eighteen snakes (13 juveniles, three adult males and two adult females, or 13 juveniles, four adult males and one adult female) were stratified on the basis of maturity and sex and randomly assigned to each of the six treatments. For each treatment, the snakes were individually weighed and randomly assigned to one of 18 positions inside the container. At the time of treatment, snakes were individually bagged in unique numbered cloth bags that corresponded to the positions inside the container and were suspended by ropes from the ceiling along six rows near the top (high, H), middle (M); and floor (low, L) positions as indicated in Fig. 1.

Liquid MB from the compressed gas cylinder was delivered via a dispenser through coiled tubing in a heated water bath to generate MB gas, which then passed through a hose into the test container. Minimum/maximum temperature and humidity were recorded for each treatment with a digital thermometer/hygrometer positioned in the rear of each container about 1.2 m from the end, 0.9 m from the right side, and 1.2 m from the floor. Possible leakage of MB was monitored with a halide flame leak detector; a self-contained breathing apparatus was worn while making leak checks. At approx. 30 min, 1 h or 2 h after MB introduction, concentrations were determined with a calibrated thermal conductivity gas analyzer (Fumiscope$^{®}$, Key Chemical Co., Clearwater, Florida, USA) from three sampling leads placed near the floor (front),

Row

6	16 M	17 H	18 L
5	13 H	14 L	15 M
4	10 L	11 M	12 H
3	7 M	8 H	9 L
2	4 H	5 L	6 M
1	1 L	2 M	3 H

Left   Center   Right

Front of Container

Fig. 1. Schematic for assignment of brown treesnakes to 18 positions within a cargo container during efficacy testing of methyl bromide as a cargo fumigant. The snakes were positioned in cloth bags inside the container that were suspended by ropes from the ceiling along six rows near the top (high, H); (middle, M); and floor (low, L) and across the left, center, and right of the containeright of the container. (not to scale).

center (middle), and top (rear) of the MB-treated container. One sampling lead was placed in the center (middle) of the control (Tl) container, but no inert carrier was introduced into the container. Concentration × time (ct) products (Monro, 1969) were calculated from the average of the MB concentrations recorded for each treatment. Measurements were recorded in Imperial, but were converted to the metric system. Dosages and concentrations tested as oz $1000\,ft^{-3}$ are equivalent to $g\,m^{-3}$ (Monro, 1969).

At the end of the MB exposure period, containers were aerated by an exhaust fan. Draeger® MB detection tubes (CH 27301, National Draeger Inc., Pittsburgh, Pennsylvania USA) were used to ensure that the containers were safe ($\leqslant 5\,ppm$), for reentry by research personnel. Snakes were returned to their cages and observed at least once daily for 10–11 days post-treatment. Post-treatment body weights were recorded for all snakes, except for T2.

Nonparametric survival analyses were conducted on treatments T2 through T6 (Kaplan and Meier, 1958) using the LIFETEST procedure in the SAS program package (SAS Institute, Inc., 1996). The Wilcoxon test was used to make comparisons of survival curves (Breslow, 1970). Mean snake weight comparisons were made using the nonparametric Kruskal-Wallis one-way analysis of variance by ranks. If this analysis indicated a significant difference at $\alpha = 0.05$, a Bonferroni-type comparison procedure based on ranks was used to find the differences which were significant (Neter et al., 1985).

3. Results

The mortality of brown treesnakes for each MB treatment is shown in Tables 1 and 2. All snakes in treatment T2 ($24\,g\,MB\,m^{-3}$) died within 18 h after exposure for 2 h Because T2 was successful, the same dose for 1 h was tested (T3); again all snakes died, but time to death was longer (Table 2). When in treatment T4 a lower dose ($12\,g\,MB\,m^{-3}$) was used for 1 h, only one of the 18 snakes died. T5 replicated T3, and all snakes died within about 9.5 days. Treatment T6 re-examined the fumigant dosage from T4, but the exposure period was extended from 1 h to 2 h, and all snakes died within about 2.5 days. Concentration × time products ranging from 22 to $44\,g\,h^{-1}\,m^{-3}$ resulted in 100% mortality of the snakes (Table 1). No mortalities or signs of toxicity occurred in the control group (Tl). Signs of toxicity noted in the snakes treated with MB were anorexia, lethargy, loss of muscle tone and loss of righting reflex. The mean minimum/maximum temperatures and RHs for all treatments were 27 °C, SD = 1 °C/ 28 °C, SD = 2 °C and 89%, SD = 2%/92%, SD = 2%, respectively.

Recorded MB concentrations ranged from 90 to 102% of the $24\,g\,MB\,m^{-3}$ nominal value (T2, T3, T5) and 92 to 100% of the $12\,g\,MB\,m^{-3}$ nominal value (T4, T6) for the duration of each treatment. MB was not detected in the control container.

Survival analyses data from treatment T4 ($12\,g\,MB\,m^{-3}$ for 1 h) was excluded because all but one snake survived, thus leaving this treatment different from all other treatments. For the overall comparison, there was a *p*-value of 0.0001, indicating a difference among the five survival curves. Treatment T2 ($24\,g\,MB\,m^{-3}$ over 2 h) provided substantially shorter survival than the other treatments, and fairly consistent results were obtained from the replicated treatments of $24\,g\,MB\,m^{-3}$ for 1 h (T3 and T5). Survival results were

Table 2
Daily post-treatment mortality of brown treesnakes fumigated with methyl bromide (MB) in simulated cargo containers

Treatment	No. of dead snakes on post-treatment day										
	1	2	3	4	5	6	7	8	9	10	11
T1 (control)											
T2 ($24\,g\,MB\,m^{-3}$, 2 h)	18										
T3 ($24\,g\,MB\,m^{-3}$, 1 h)	8	5	2		1	1					1
T4 ($12\,g\,MB\,m^{-3}$, 1 h)				1							
T5, ($24\,g\,MB\,m^{-3}$, 1 h)	7	10								1	
T6 ($12\,g\,MB\,m^{-3}$, 2 h)	17		1								

Table 1
Dosage, exposure time, concentration × time (ct) product, and mortality of brown treesnakes fumigated with methyl bromide in simulated cargo containers

Treatment no.	Dosage ($g\,m^{-3}$)	Exposure time (h)	ct product ($g\,h^{-1}\,m^{-3}$)	Mortality	
				Dead/treated	Days to 100%
T1	Control	2	0	0/18	NA
T2	24	2	44	18/18	<1
T3	24	1	23	18/18	10.5
T4	12	1	11	1/18	NA
T5	24	1	24	18/18	9.5
T6	12	2	22	18/18	2.5

similar for T3 and T5 ($24\,\mathrm{g\,MB\,m^{-3}}$ for 1 h) and T6 ($12\,\mathrm{g\,MB\,m^{-3}}$ for 2 h).

The difference between post- and pre-test weight was calculated for all the snakes in each treatment except T2. The Kruskal-Wallis test showed significant ($p = 0.002$) differences among the weights. A Bonferroni-type comparison procedure was used to find the differences which were significant. The results indicated that the weight differences between treatment groups were significantly different from zero for T1 and T3, T1 and T5, and T1 and T6. This is not surprising since T1 was the control in which no signs of toxicity were noted, whereas 100% mortality occurred in the T3, T5, and T6 treatments.

4. Discussion

This study demonstrated that methyl bromide, at or below many currently registered application rates, consistently kills brown tree snakes in cargo containers. The application rate of $24\,\mathrm{g\,MB\,m^{-3}}$ (1.5 lb MB $1000\,\mathrm{ft^{-3}}$) for 2 h (T2) was the treatment schedule of choice because it resulted in 100% mortality in a relatively short time, and would allow for under-treatment during commercial fumigation operations (Table 1).

An interesting observation of this study was the apparent sensitivity of snakes to MB fumigation, as indicated by the results of the $12\,\mathrm{g\,MB\,m^{-3}}$ for 2 h treatment (T6). Of the numerous EPA-registered fumigation schedules for MB, all use at least the effective rates tested in the study, usually for much longer exposure periods (Great Lakes Chemical Corporation, 1987). This suggests that most cargo fumigated with MB should not contain live snakes upon reaching its destination. However, in the event that snakes in cargo do not die within the time period of shipping, the data from T6 would suggest that they will not live long upon arrival at the cargo destination. Data from T3 and T5 would suggest that even if they are alive upon arrival, they will eventually die.

On 4 November 1994, the US EPA approved addition of the brown treesnake to the MB label (EPA Registration No.5785-41, Meth-O-Gas® Q Commodity Fumigant, 100% MB). Thus, MB is now available for use as part of an integrated pest management system for controlling these environmentally disruptive snakes (Engeman and Vice, 2001). MB would be useful in cargo that is impractical or impossible to search to quickly ensure death of brown treesnakes without significant disruption of commerce. Another positive benefit is that MB treatment would also be effective against other pest and environmental disruptors, such as insects and rodents.

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